```
hFRS2:      1 MGSCCSCPDKDTVPDNHRNKFKVINVDDDGNELGSGIMELTDTELILYTRKRDSVKWHYL
mFRS2:        M-----------------------------------V-----------------------
hFRS3:      1 MGSCCSCLNRDSVPDNHPTKFKVTNVDDEGVELGSGVMELTQSELVLHLHRREAVRWPYL
mFRS3:        M---W---D------H-------------------------------Q---------
              **** **    * ** **   **** **** * ***** ****  ** *     *  * * **

hFRS2:     61 CLRRYGYDSNLFSFESGRRCQTGQGIFAFKCARAEELFNMLQEIMQNNSINVVEEPVVE
mFRS2:        ------------------------------------------------------------
hFRS3:     61 CLRRYGYDSNLFSFESGRRCQTGQGIFAFKCSRAEEIFNLLQDLMQCNSINVMEEPVIIT
mFRS3:        ---------------------------------D----------------T--------
              ********************************* ***  ** **  ** ***** ****

hFRS2:    120 RNNHQTELEVPRTPRTPTTPGFAAQNLPNGYPRYPSFGDASSHPSSRHPSVGSARLPSVG
mFRS2:        -SS-----------------LG-------------------------------------
hFRS3:    121 RNSHPAELDLPRAP QPPNALGYTVSSFSNGCP    GEGPRFSAPRRLSTSSLRHPSLG
mFRS3:        -S---P------G-P--AGYTVSGF-NGFP---    ----------P---------P-
              *  *  **  ** *  *                   *          *  *  * * ** *

                                    Grb2
hFRS2:    180 EESTHPLLVAEEQVHTYVNTTGVQEERKNRTSVHVPLEARVSNAESSTPKEEPSSIEDRD
mFRS2:        ----------------------------A-----P---------N-------NP----
hFRS3:    176 EESTHALIAPDEQSHTYVNTPASEDDHRRGRHCLQPLPE  GQAPFLPQARGP    DQRD
mFRS3:        -----T---SE----------TGDE-G-S ---------  -RV-LPA-TQ-S    ----
              ** ** *    ** ******                  *                    **

hFRS2:    240 PQILLEPEGVKFVLGPTPVQKQLMEKEKLEQLGRDQVSGSGANNTEWDTGYDSDERRDAP
mFRS2:        --V--K----R-------------------K-P------G---------------V-
hFRS3:    231 PQVFLQPGQVKFVLGPTPARRHMVKCQGLCPSLHDPPHHNNNNEAP            SECP
mFRS3:        ---L----------------QVM---S---GMQ-------- -G-            ----
              **  * *  * *******          *     *                      *

                  Grb2                                          Grb2
hFRS2:    300 SVNKLVYENINGLSIPSASGVRRGRLTSTSTSDTQNINNSAQRRTALLNYENLPSLPPVW
mFRS2:        P-------------------------------------P---------------
hFRS3:    281 AQPKCTYENVTG         GLWRGAGWRLSPEEPGWNGLAHRRAALLHYENLPPLPPVW
mFRS3:        ----------S-         --QQ----------R--S--------------------
                 *  ***  *                                ** *** ***** *****

                                                Grb2
hFRS2:    360 EARKLSRDEDDNLGPKTPSLNGYHNNLDPMHNYVNTENVTVPASAHKIEYSR
mFRS2:        ------------------------------------------D--K
hFRS3:    333 ESQAQQLGGEAGDDGDSRDGLTPSSNGFPDGEEDETPLQKPTSTRAAIRSHGSFPVPLTR
mFRS3:        ---G--P-------------------------------------SA---SG-------
              *                          *  *       *

                                    SH-PTP2
hFRS2:    412 RRDCTPTVFNFDIRRPSLEH RQLNYIQVDLEGGSDSDNPQTPKTPTTPLPQTPTRR
mFRS2:        ------------------- ---------------------------------
hFRS3:    393 RRG SPRVFNFDFRRPGPEPPRQLNYIQVELKGWGGDRPKGPQNPSSPQAPMPTTHPARS
mFRS3:        --- -----------Q-------------------TA---------VSG--G--P--V--
              **   * ***** **   *  ******** * *                    *

                  SH-PTP2
hFRS2:    468 TELYAVIDIERTAAMSNLQKALPRDDGTSRKTRHNSTDLPM 508
mFRS2:        ------------------------------------------
hFRS3:    452 SDSYAVIDLKKTVAMSNLQRALPRDDGTARKTRHNSTDLPL 492
mFRS3:        ----------A---D----------AV-----------
                 *****   * *** ** *******  **********
```

FIGURE 1

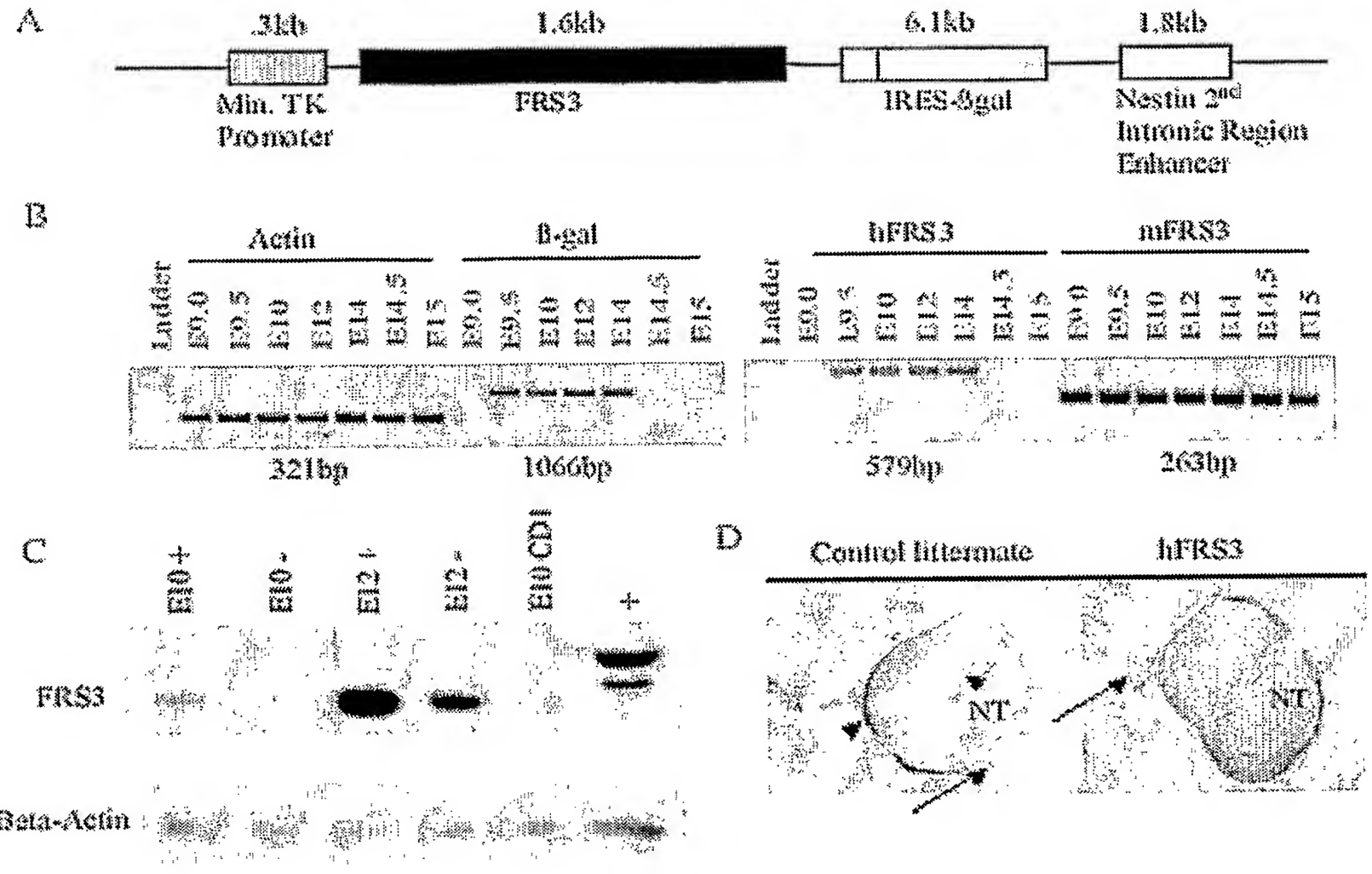

A
3kb
1.6kb
6.1kb
1.8kb
Min. TK Promoter
FRS3
IRES-βgal
Nestin 2nd Intronic Region Enhancer
B
Actin
β-gal
hFRS3
mFRS3
Ladder
E9.0
E9.5
E10
E12
E14
E14.5
E15
321bp
1066bp
579bp
263bp
C
E9.0 +
E9.0 -
E12 -
E12 +
E10 CDI
+
FRS3
Beta-Actin
D
Control littermate
hFRS3
NT
NT

FIGURE 2

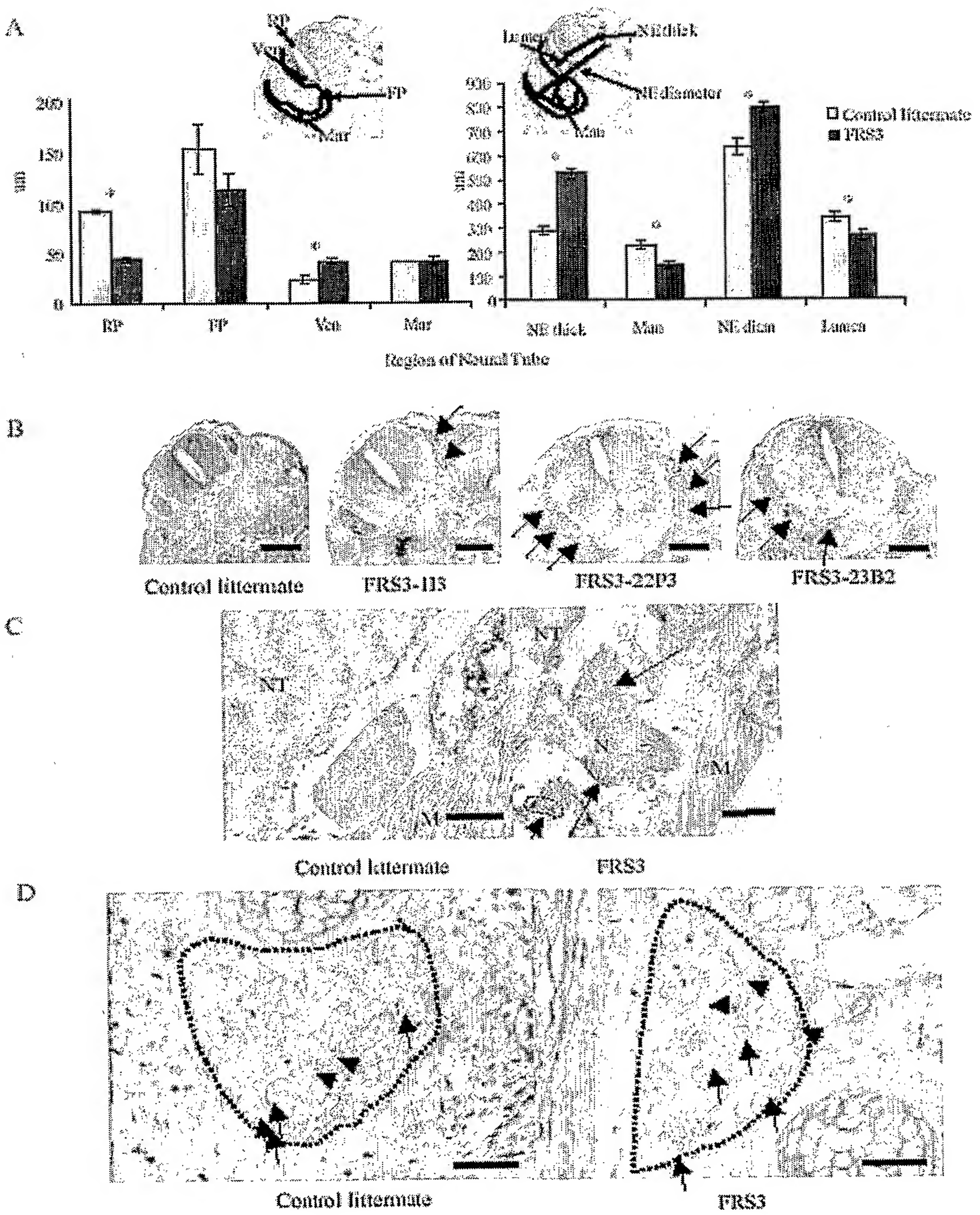
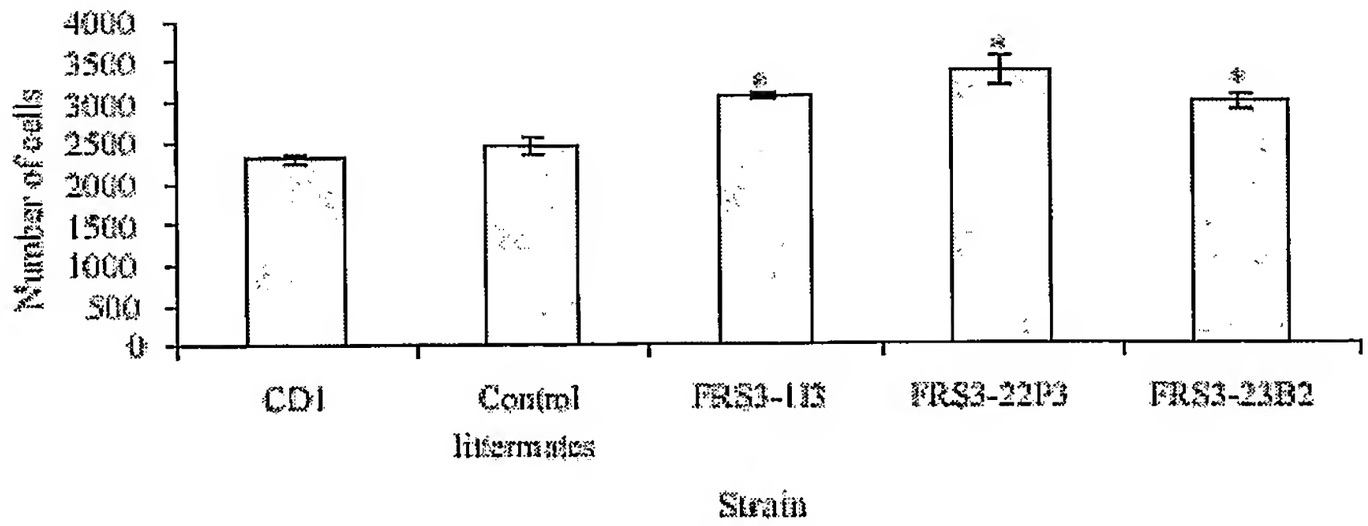

A
BP
FP
Ven
Mar
Region of Neural Tube
NE thick
Lumen
NE diameter
Mes
Control littermate
FRS3
NE thick
Mes
NE diam
Lumen
B
Control littermate
FRS3-11B
FRS3-22P3
FRS3-23B2
C
NT
M
Control littermate
FRS3
D
Control littermate
FRS3

FIGURE 3

A


Number of cells
4000
3500
3000
2500
2000
1500
1000
500
0
CD1
Control littermates
FRS3-11B
FRS3-22P3
FRS3-23B2
Strain

B

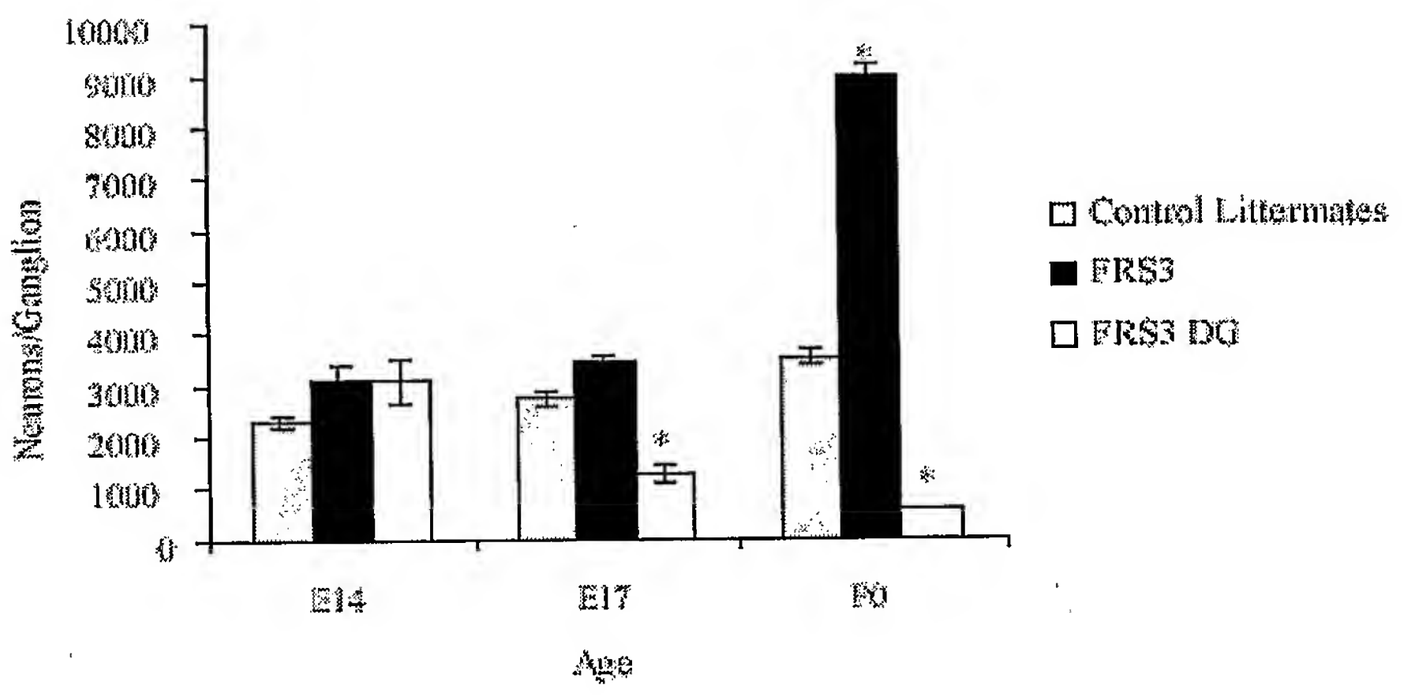

Neurons/Ganglion
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
E14
E17
P0
Age
Control Littermates
FRS3
FRS3 DG

FIGURE 4

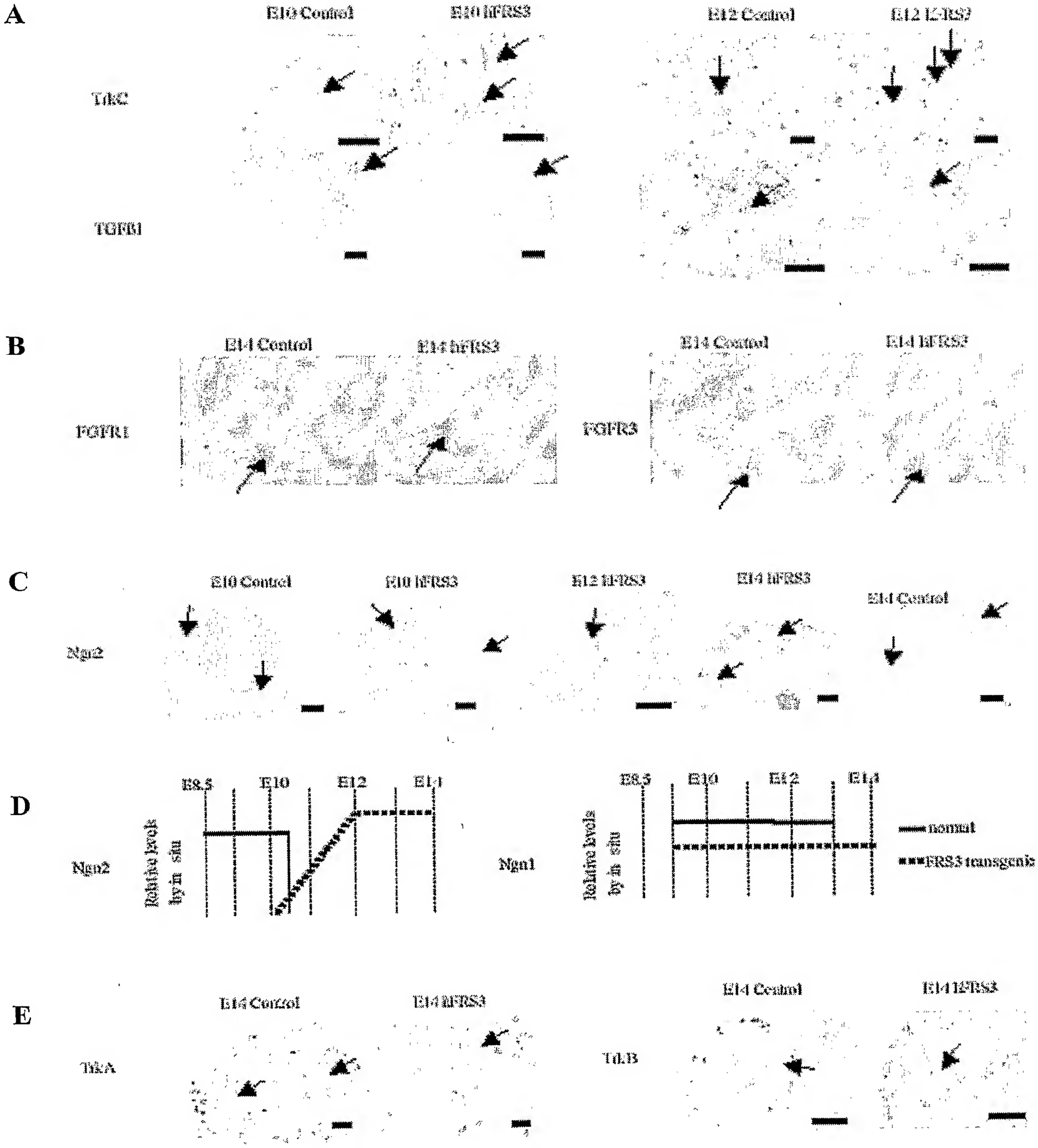

A
E10 Control
E10 hFRS3
E12 Control
E12 hFRS3
TrkC
TGFB1
B
E14 Control
E14 hFRS3
FGFR1
E14 Control
E14 hFRS3
FGFR3
C
E10 Control
E10 hFRS3
E12 hFRS3
E14 hFRS3
E14 Control
Ngn2
D
E8.5
E10
E12
E14
Relative levels by in situ
Ngn2
Ngn1
E8.5
E10
E12
E14
Relative levels by in situ
normal
FRS3 transgenic
E
E14 Control
E14 hFRS3
TrkA
E14 Control
E14 hFRS3
TrkB

FIGURE 5

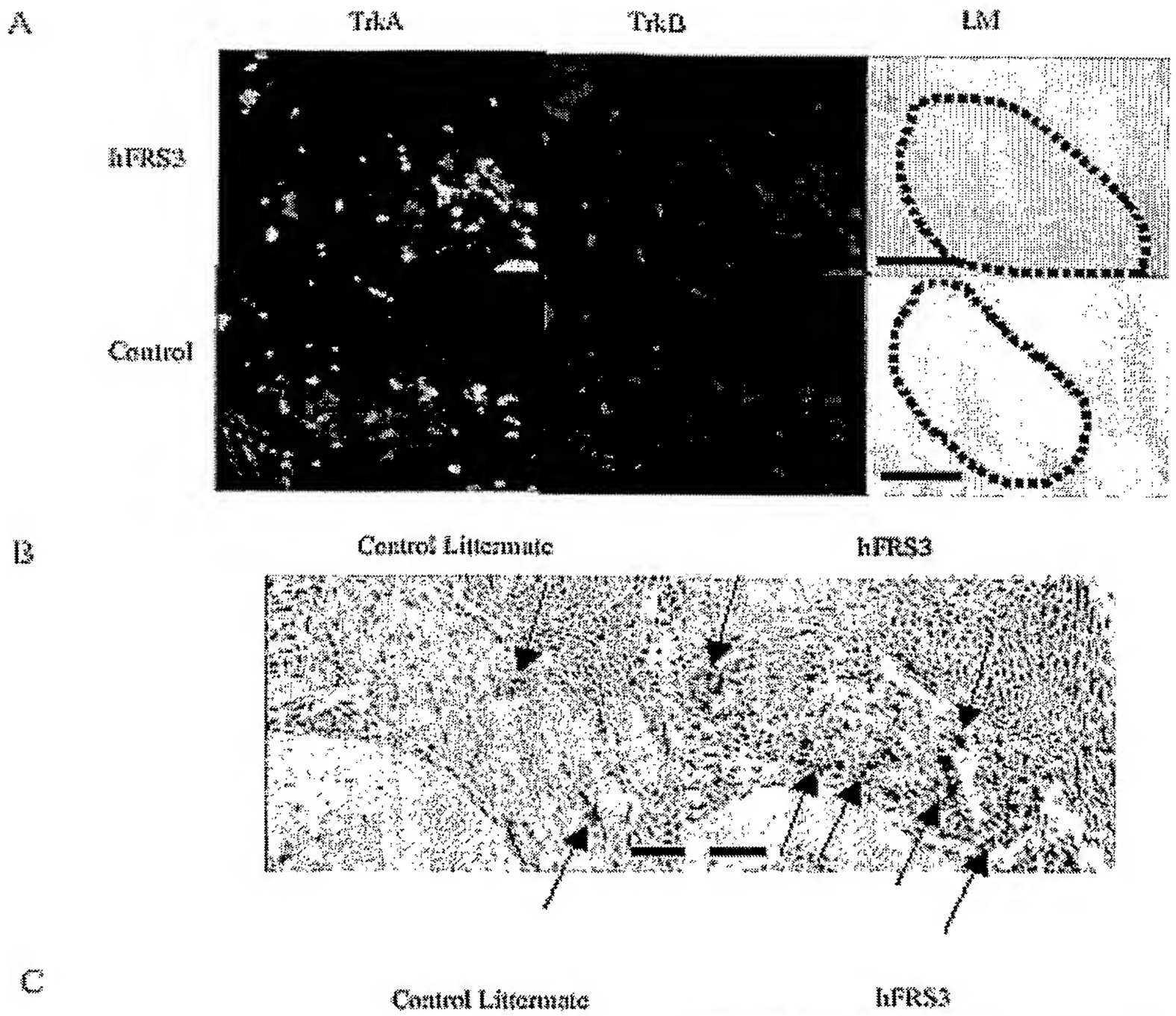

A
TrkA
TrkB
LM
hFRS3
Control
B
Control Littermate
hFRS3
C
Control Littermate
hFRS3
NT
NT

FIGURE 6

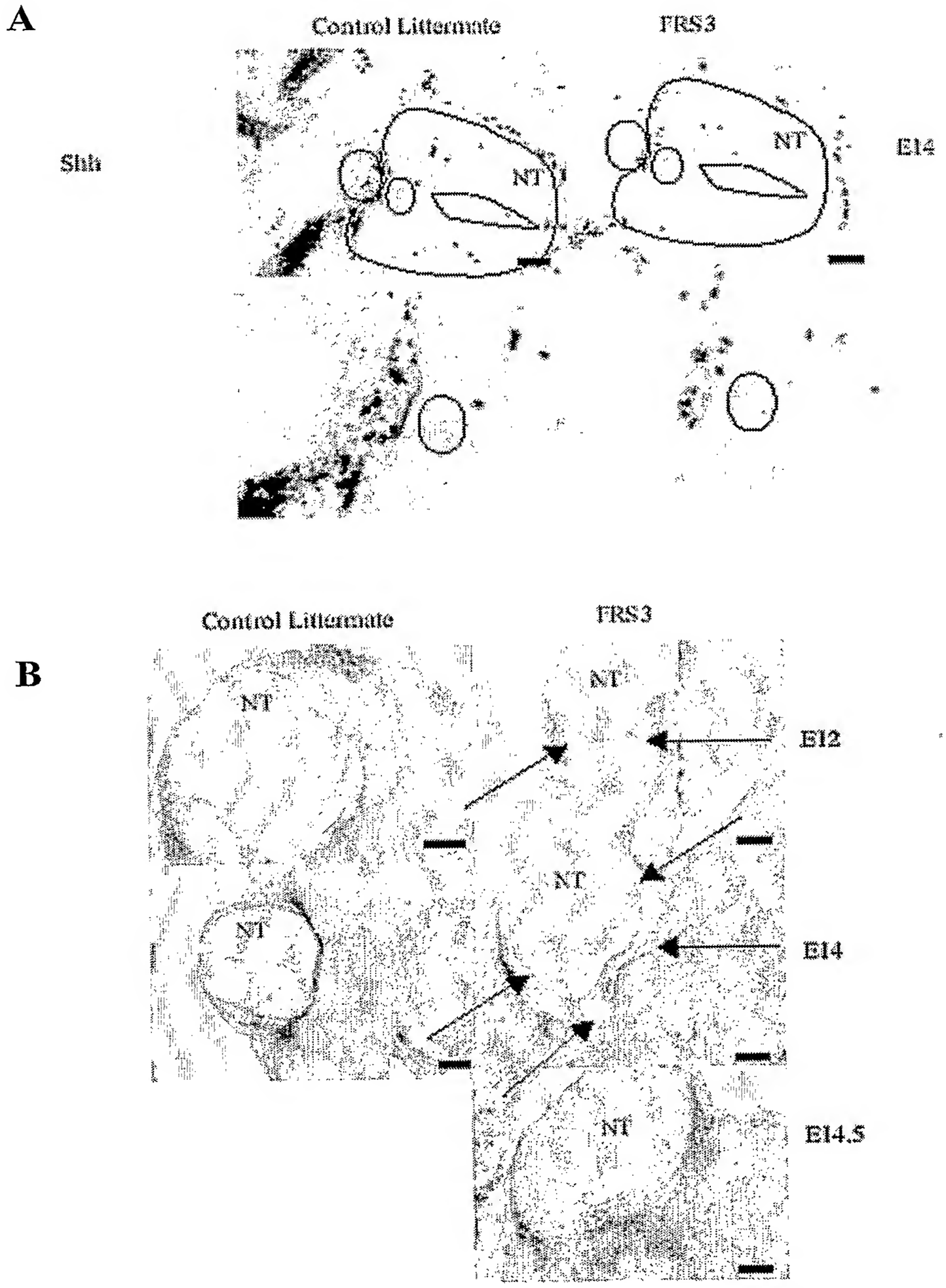
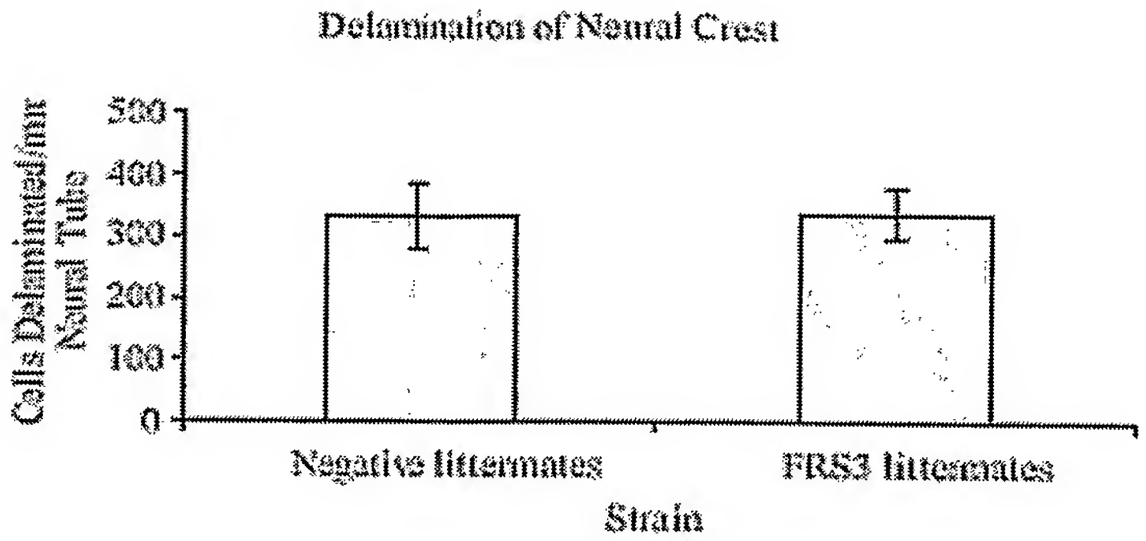
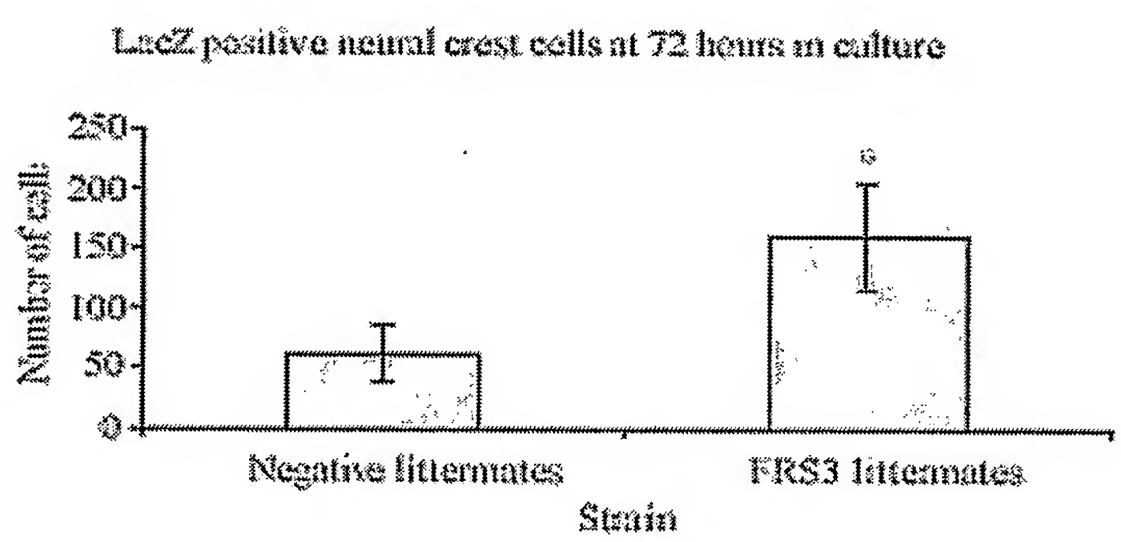

A
Control Littermate
FRS3
Shh
NT
NT
E14
B
Control Littermate
FRS3
NT
NT
E12
NT
NT
E14
NT
NT
E14.5

FIGURE 7

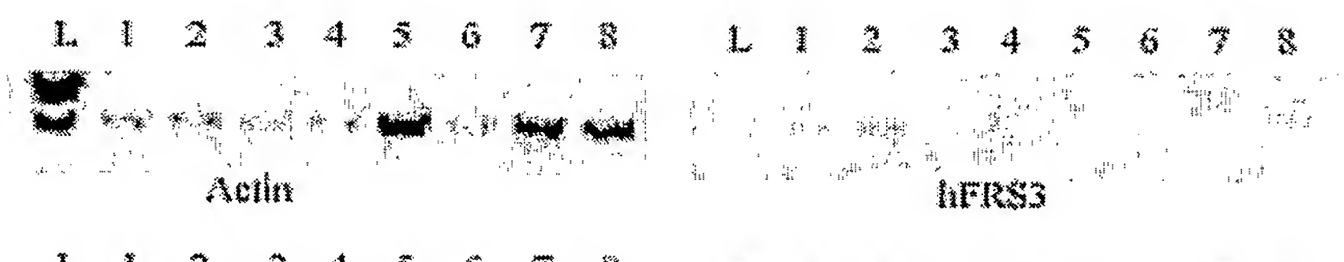
A



B



C



D

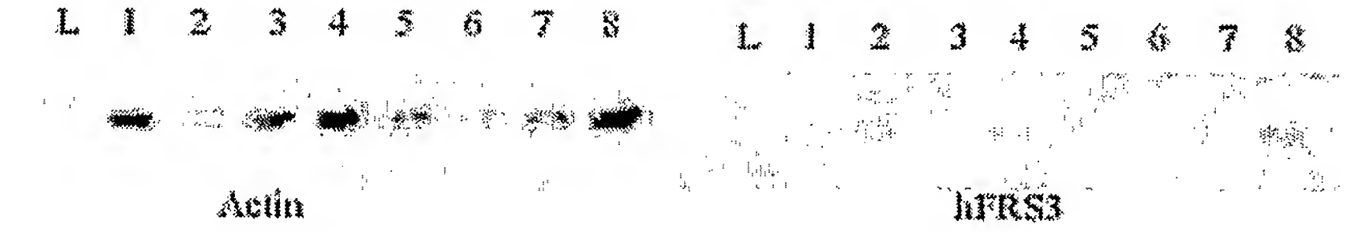
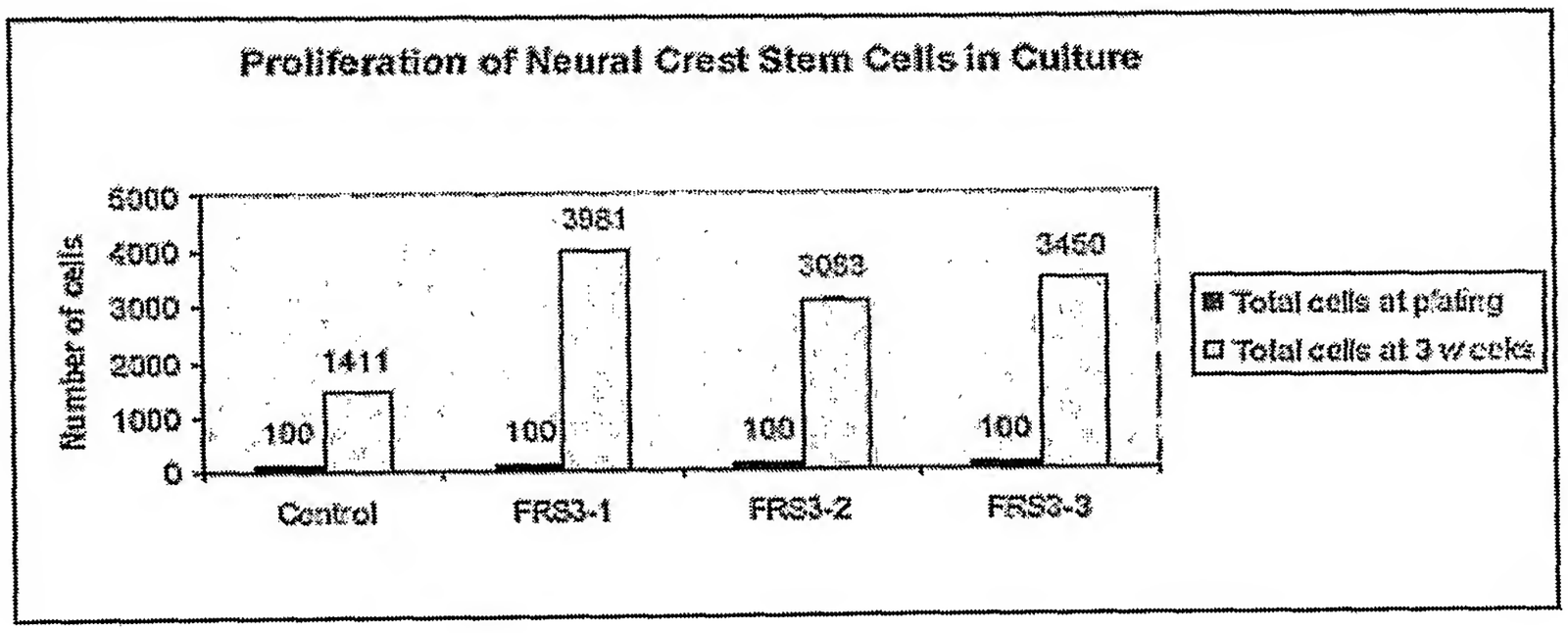


FIGURE 8

A


Proliferation of Neural Crest Stem Cells in Culture
Number of cells
5000
4000
3000
2000
1000
0
100
1411
100
3981
100
3083
100
3450
Control
FRS3-1
FRS3-2
FRS3-3
Total cells at plating
Total cells at 3 weeks

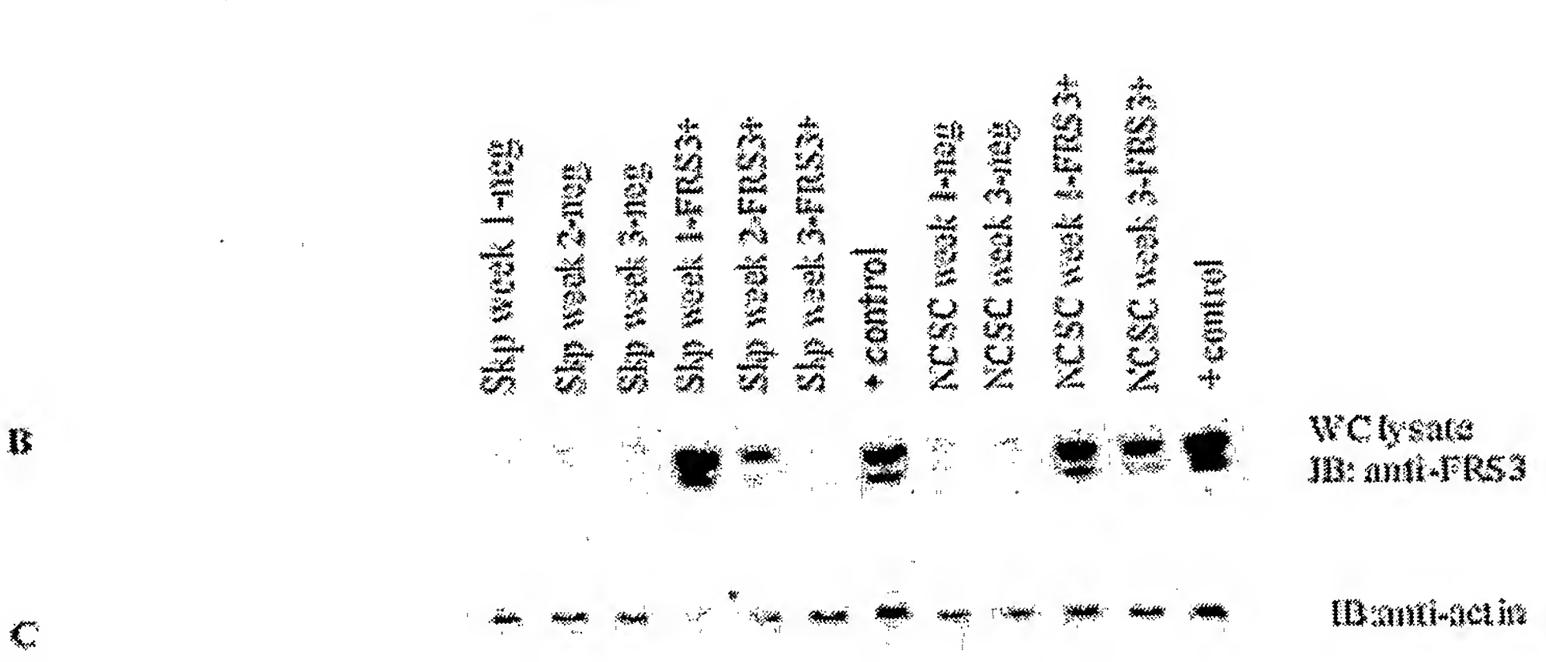
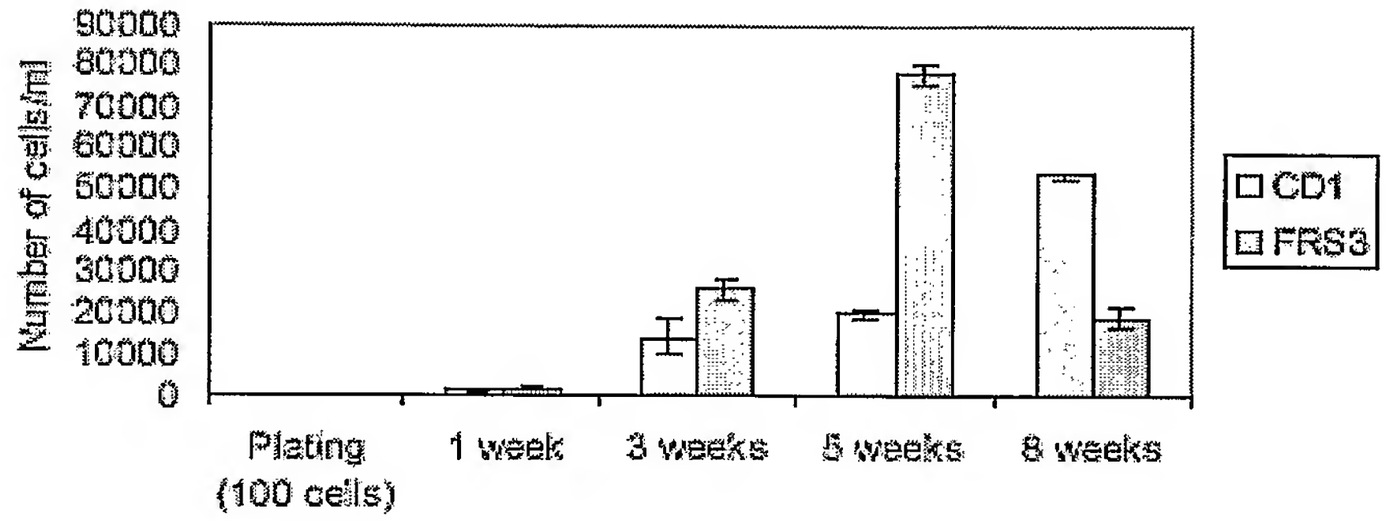

Skp week 1-neg
Skp week 2-neg
Skp week 3-neg
Skp week 1-FRS3+
Skp week 2-FRS3+
Skp week 3-FRS3+
+ control
NCSC week 1-neg
NCSC week 3-neg
NCSC week 1-FRS3+
NCSC week 3-FRS3+
+ control
B
WC lysate
IB: anti-FRS3
C
IB:anti-actin

FIGURE 9

A


Number of cells/ml
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Plating (100 cells)
1 week
3 weeks
5 weeks
8 weeks
CD1
FRS3

B

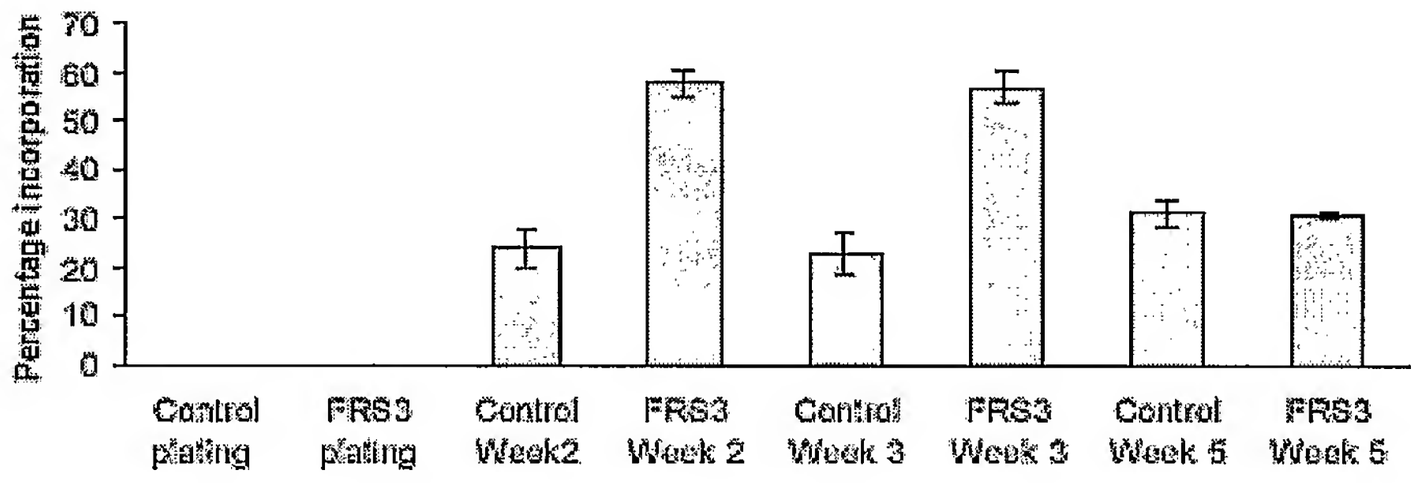

Percentage incorporation
70
60
50
40
30
20
10
0
Control plating
FRS3 plating
Control Week2
FRS3 Week 2
Control Week 3
FRS3 Week 3
Control Week 5
FRS3 Week 5

FIGURE 10